1: Biotechniques. 1999 Oct;27(4):728-32.

Links

Co-transfected SV40 origin of replication activates expression from SV40 promoterless constructs.

Flint A, Kaluz S, Kaluzova M, Sheldrick L, Fisher P, Derecka K.

University of Nottingham, Loughborough, Leics, England, UK.

Co-transfection with expression plasmids is widely used to control DNA uptake efficiency in transient transfection experiments. However, a number of problems have been associated with their use. Here, we describe the activation of expression of constructs not containing the simian virus 40 (SV40) origin of replication (ori) by co-transfection in COS-7 cells with plasmids containing the SV40 ori. This effect has consequences for the use of such plasmids to control transfection efficiency.

PMID: 10524314 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1978 Apr;75(4):1929-33.

Transformation of yeast.

Hinnen A, **Hicks JB**, **Fink GR**.

A stable leu2- yeast strain has been transformed to LEU2+ by using a chimeric ColE1 plasmid carrying the yeast leu2 gene. We have used recently developed hybridization and restriction endonuclease mapping techniques to demonstrate directly the presence of the transforming DNA in the yeast genome and also to determine the arrangement of the sequences that were introduced. These studies show that ColE1 DNA together with the yeast sequences can integrate into the yeast chromosomes. This integration may be additive or substitutive. The bacterial plasmid sequences, once integrated, behave as a simple Mendelian element. In addition, we have determined the genetic linkage relationships for each newly introduced LEU2+ allele with the original leu2- allele. These studies show that the transforming squences integrate not only in the leu2 region but also in several other chromosomal locations.

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Transcriptional regulation in the yeast *GAL* gene family: a complex genetic network

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ABSTRACT **Regulation of the *GAL* structural genes in the yeast *Saccharomyces cerevisiae* is implemented by the products of *GAL*-specific (*GAL4*, *GAL80*, *GAL3*) and general (*GAL11*, *SWI1*, *2*, *3*, *SNF5*, *6*, numerous glucose repression) genes. Recent work has *1)* yielded significant new insights on the DNA binding and transcription activation/Gal80 protein binding functions of the Gal4 activator protein, *2)* described the characterization of purified Gal4 protein-Gal80 protein complexes, *3)* deconvoluted the multiple and complex glucose repression pathways acting on *GAL* genes, *4)* suggested a new mechanism for the Gal3 protein-mediated induction of *GAL* structural gene expression, *5)* introduced Gal1 protein, a structural gene product, into the regulation scheme, and *6)* extended our already substantial understanding of *GAL* regulatory gene control. The mechanisms which control structural and regulatory gene expression in the *GAL* family are compared and *GAL* structural/regulatory gene chromatin structure is discussed.— Lohr, D., Venkov, P., Zlatanova, J. Transcriptional regulation in the yeast *GAL* gene family: a complex genetic network. *FASEB J.* 9, 777–787 (1995)**

Key Words: Gal4p • galactose • nucleosomes • regulatory gene control • S. cerevisiae

BACKGROUND

The *GAL* gene family, the set of structural and regulatory genes (**Table 1**) that enable cells to utilize galactose as a carbon source, is one of the best–studied systems in the budding yeast *Saccharomyces cerevisiae*, a major model for the study of eukaryotic regulation. The *GAL* genes are strongly regulated at the transcriptional level via carbon source. Because they have been extensively analyzed by classical genetic techniques and by biochemical approaches, which have elucidated some of the structural features underlying the genetic relationships, these genes can provide novel and detailed insights on eukaryotic transcriptional regulation, both at the individual gene level and for family interrelationships. *GAL* regulation was comprehensively reviewed in 1987 (1) and, along with other yeast carbon- and phosphate-utilization gene families, in 1992 (2). We review the current status of *GAL* regulation, emphasizing topics not extensively discussed recently (2), and a biochemical viewpoint. Some models are suggested, which are intended mainly as a stimulus to further experiments. We apologize to those whose work is not directly cited due to space limitations.

The *GAL* structural genes

GAL2, *GAL1-GAL7-GAL10*, and *MEL1* are traditionally considered to be the *GAL* structural genes (1). Their gene products transport galactose into cells, convert intracellular galactose to the glycolytic substrate glucose-1-phosphate, and provide a galactosidase activity (Table 1). *GAL5*, which encodes an enzyme (phosphoglucomutase) needed in galactose utilization (Table 1) and in other pathways, also demonstrates some aspects of *GAL* regulation (2). The *GAL* structural genes can be found in three major types of regulated states, dependent on carbon source (Table 1): inactive-repressed (glucose); inactive, poised for induction (glycerol); active, induced to high-level expression (galactose). Expression in galactose is extremely robust; the *GAL1*, *-7*, and *-10* mRNAs each become 0.25–1% of total polyadenylated mRNA (1). Thus, the mechanisms that activate these genes must be very powerful. On the other hand, *GAL1*, *-2*, *-7*, and *-10* are not detectably expressed in glucose or glycerol, so the mechanisms that inhibit their expression under these conditions must be highly effective. It is this efficiency and precision of regulation that makes the *GAL* genes such an attractive model system.

The basic regulatory strategy

Regulation in the *GAL* family is implemented by a network of activating and repressing activities encoded by both

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TABLE 1. *The GAL gene family*

Gene	Expression in Gl	Gly	Gal	Induction Fold[a]	#UAS_G[b]	Function
A) STRUCTURAL						
GAL2	0	0	+	>1000	2	Gal transport
GAL1	0	0	+	>1000	4[c]	Gal→Gal-1-P
GAL7	0	0	+	>1000	2	Gal-1-P→Gl-1-P
GAL10	0	0	+	>1000	4[c]	UDP-Gl ⇄ UDP-Gal (Gal10p)
MEL1	0	+	+	>100	1	Galactosidase
(GAL5)	0	+	+	3-4		Gl-1-P→Gl-6-P
B) REGULATORY						
GAL4	+	+	+	<1	0	Transcription Activation
GAL80	+	+	+	5-10	1	Inhibition of Gal4p
GAL3	0	+	+	3-5	1	Release of Gal4p Inhibition

[a]Galactose versus glycerol. [b]Consensus sequence: 5'CGGAGGAC T/A GTCCTCCG3'. [c]Shared between *GAL1* and *GAL10*. Expression in various carbon sources is qualitatively indicated (Gl = glucose, Gly = glycerol, Gal = galactose). The expression data are taken from references cited in the text. The UAS_G numbers and the consensus sequence are from reference 1. Throughout the paper *GAL2* etc. refers to the gene. Gal2p etc. to the product of that gene. The parentheses around *GAL5* refer to its partial resemblance to a *GAL* structural gene.

GAL-specific and general genes (**Fig. 1**). The specific regulatory genes, *GAL3*, *GAL4*, and *GAL80*, provide the major structural gene regulators (1, 2). Gal4p[2] is required for the powerful galactose-induction of *GAL* gene expression. Gal4p activates transcription through a specific carboxy–terminal domain while bound to the promoter via its amino terminus (see Specific Regulatory Factors). Gal80p binds to and masks the Gal4p transcription activation function in nongalactose carbon sources (glucose, glycerol), even if Gal4p is bound to the promoter (glycerol). Gal3p mediates the galactose-induced release of Gal80p inhibition of Gal4p (see Specific Regulatory Factors). In glucose, several diverse mechanisms implement severe repression (see Structural Gene Expression/Regulation). *GAL4*, *GAL80*, and *GAL3* expression is also regulated. Studies of their control provide insight on this little-known aspect of eukaryotic regulation (see Regulation of the *GAL*–Specific Regulatory Genes).

The UAS_G

The UAS_G: the DNA element that mediates galactose-induction of expression by serving as the Gal4p binding site. The major *GAL* promoter element is the UAS_G (1). It mediates the galactose-induction of both structural and regulatory *GAL* gene expression (Table 1) and can confer galactose inducibility on a heterologous gene, a property that has been widely used for heterologous expression (3). UAS_G elements range from 11 to 14 matches to a 17 bp consensus sequence (1; Table 1). They are probably not all functionally equivalent (1), differing in intrinsic effectiveness and in their interdependence on one another (4). The UAS_G is the DNA binding site of Gal4p (1, 2). On the structural genes like *GAL1-10* and *GAL2*, Gal4p protects the UAS_G strongly in galactose, where the genes are active, and in glycerol (1, 5), where they are inactive but poised for ex-

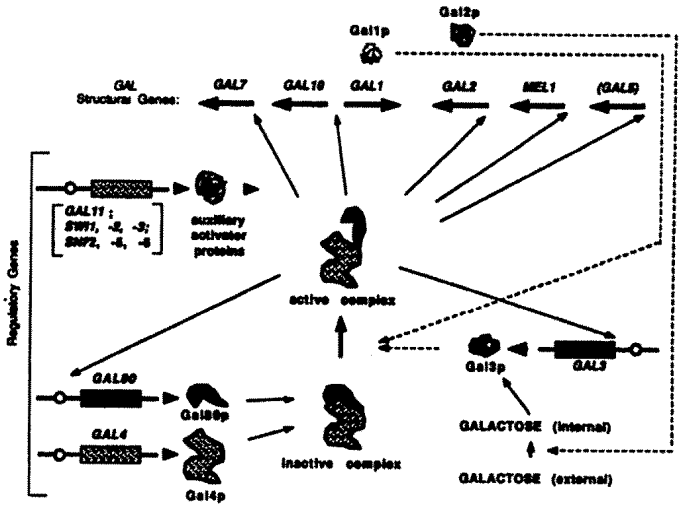

Figure 1. Galactose induction pathways. This figure summarizes the interrelationships and interactions among the *GAL* regulatory/structural genes and regulatory/structural proteins and global activation factors. *GAL* structural genes are at the top; the large arrows denote the transcription units for the various genes. Regulatory genes are shown on the left and right sides. Arrows (thin solid or dotted lines) refer to the regulation flow within the family.

[2]Abbreviations: Gal4p, Gal4 protein; Gal3p, Gal3 protein; Gal80p, Gal80 protein; UAS_G, upstream activation sequence; UES, upstream essential sequence; URS_G, upstream repression sequence; ID, inhibitory domain; GRD, glucose response domain; AH, amphipathic helix.

TABLE 2. *Gal4p binding to the UAS$_G$*[a]

Gene	Glucose	Glycerol	Galactose
GAL1-10	no	strong	strong
GAL80	no	weak-moderate	strong

[a]As judged by degree of in vivo or in situ footprint protection (1, 6).

pression (see Structural Gene Expression/Regulation). The single *GAL80* UAS$_G$ is protected as strongly as any of the *GAL1-10* UAS$_G$ in galactose, and thus is capable of a strong Gal4p interaction, but it is less protected in glycerol (6; **Table 2**). These results raise the possibility that the intrinsic affinity of Gal4p for UAS$_G$ elements may be lower in glycerol than in galactose. The maintenance of a strong Gal4p/UAS$_G$ interaction on *GAL1-10* and *GAL2* in glycerol can be explained by cooperative binding effects associated with multiple UAS$_G$ elements (6; see Specific Regulatory Factors). Gal4p does not bind to any UAS$_G$ in glucose (1, 5, 6; Table 2).

The UAS$_G$ does not act like a traditional enhancer. The variation in UAS$_G$ locations, from ~100 to 400 bp upstream of the transcription start site in the various *GAL* genes (1), led to the suggestion that UAS$_G$ elements are enhancers. However, the UAS$_G$ can only function from upstream of a gene, its effective range is much more restricted than that of a typical enhancer (1) and a single element cannot operate bidirectionally (7).

Other activating elements

DNA elements that can activate transcription independently of *GAL4* or galactose (GAE; see Fig. 3) were detected on the *GAL1-10* intergenic region (8). Their activity is normally silenced by nearby negative "operator" elements (8). *GAL4* and *GAL80* also contain Gal4p-independent activating elements (see Regulation of the *GAL*–Specific Regulatory Genes).

THE SPECIFIC REGULATORY FACTORS

Gal80p

The induction response region of Gal80p is surrounded by the regions involved in the inhibition of Gal4p. The inhibition function is associated with most of the Gal80p protein, residues 1-321 and 341-423 (9; **Fig. 2**). This large region may also contain other determinants. The Gal80p region that responds to the galactose-induction signal includes residues 322-340 and lies between the regions that inhibit Gal4p. Gal80p is targeted to the nucleus by specific residues (Fig. 2).

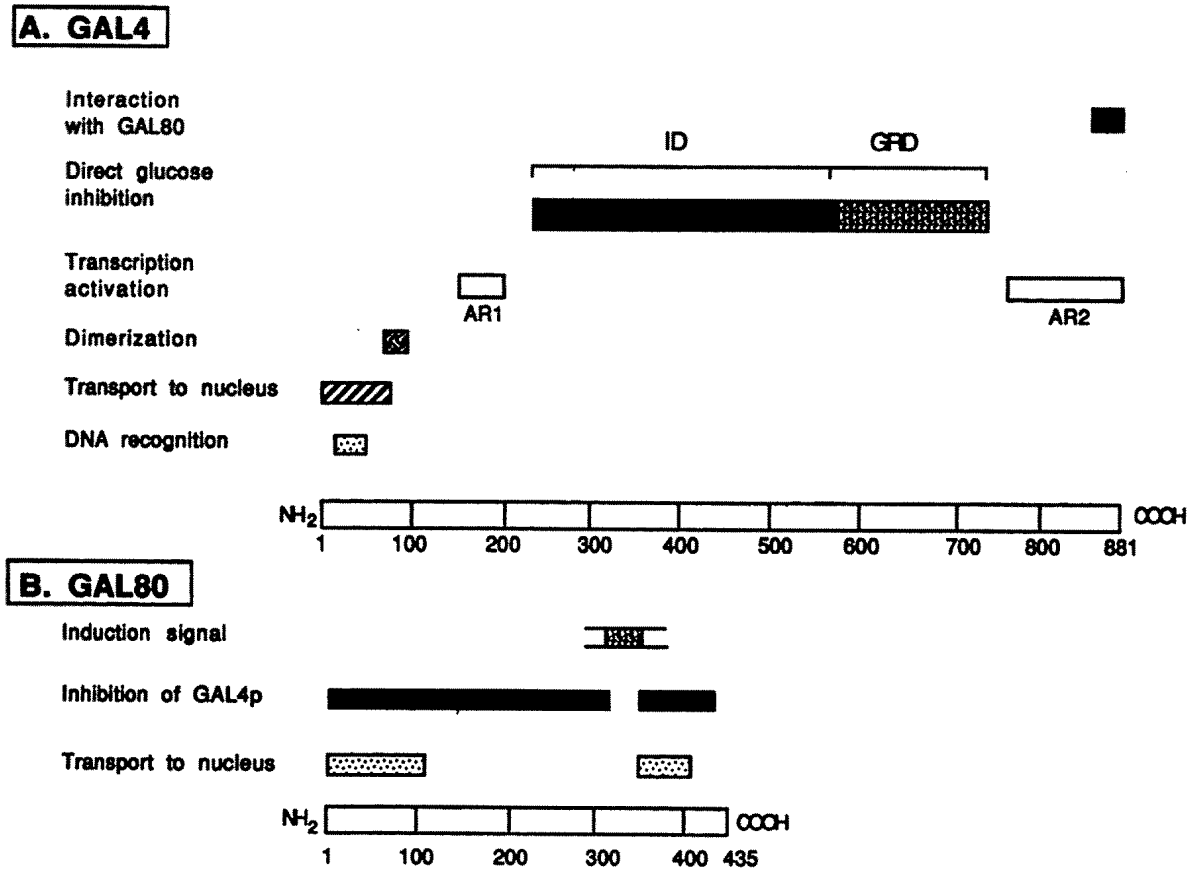

Figure 2. Domain organization of Gal4p and Gal80p. The various functional domains in the GAL4 and GAL80 regulatory proteins are located. Abbreviations for Gal4p: ID, inhibitory domain; GRD, glucose response domain; AR, activation region.

Gal4p

The 881 residue polypeptide chain of Gal4p contains several functional domains (1, 2; Fig. 2). Because of its large size and isolation difficulties (see below), most analyses have focused on particular regions of *GAL4* or fragments of Gal4p.

DNA binding

The DNA binding domain is a C_6 zinc cluster that interacts specifically with CGG triplets at the UAS_G termini. The DNA binding domain of Gal4p is localized to residues 14–57 in the amino terminus (2) and was thought to be a zinc finger (1). However, NMR analyses of various amino-terminal Gal4p fragments (2) and X-ray crystal structure determination of a consensus UAS_G/Gal4p (aa 1-65) complex (10) find that the binding module is a "C_6 zinc cluster" in which two Zn^{2+} ions are coordinated to six cysteine residues, with the rest of the amino acid 8-40 region folded around the Zn_2Cys_6. Gal4p binds the UAS_G as a dimer (1, 2). In this complex, each C_6 zinc cluster makes base-specific major groove contacts with a terminal CGG triplet (Table 1), the most conserved nucleotides in the UAS_G (1), and phosphate contacts with adjacent DNA backbone regions (10). A linker, Gal4p residues 41–49, connects the zinc cluster to the dimer interface, which is a parallel coiled coil formed by amphipathic α-helices (residues 50–64) from each monomer. Amino acid residues in the linker and adjacent part of the coiled coil contact the DNA backbone within the UAS_G, suggesting they could contribute to UAS_G recognition/binding. Genetic and biochemical data concur (1, 2, 11, 12). At least in part, this recognition appears to involve a proper "fit" of the protein to the length of the central DNA region between CGG termini (12). A whole class of C_6 zinc cluster, fungal transcription activators use this mechansim to distinguish their particular DNA binding sites (12).

The Gal4p/UAS_G interaction is intrinsically moderate in strength and specificity; multimeric interactions and coordinate binding of other proteins may enhance these properties. The dissociation constant, K_d, for Gal4p binding to *GAL1-10* DNA containing all four UAS_G elements is 2×10^{-9} M (13), reflecting a moderate affinity. Binding to a single consensus UAS_G was estimated to be even weaker, by two- to threefold. However, Gal4p fragments bind to a consensus UAS_G up to 10-fold more strongly than to a natural UAS_G (4). Thus, Gal4p affinities could differ by up to 2–3 × 10-fold for UAS_G on *GAL* genes with single vs. multiple elements. Such significant differences can explain the in situ footprinting result (see Background), which finds weaker protection of the single GAL80 UAS_G than of the multiple UAS_G on *GAL1-10* and *GAL2* in glycerol. The source of this affinity difference could involve the Gal4p dimerization domain that lies adjacent to the DNA binding domain (see Fig. 3). This hydrophobic region is suggested to strengthen the basic Gal4p dimeric structure on a UAS_G (2), but it could also allow oligomeric contacts on promoters with multiple elements, thus enhancing the strength and cooperativity of Gal4p binding on these promoters. The potential for oligomeric contacts plus the basic dimeric nature of Gal4p/UAS_G binding should make this interaction very sensitive to Gal4p concentrations, an important feature in GAL regulation models (see Regulation of the *GAL*–Specific Regulatory Genes).

Binding to DNA containing (four) UAS_G elements is only ~1000-fold stronger than binding to DNA lacking UAS_G (13). This is consistent with the limited Gal4p interaction with specific UAS_G nucleotides, but given this moderate specificity and affinity, how does Gal4p find the UAS_G in vivo? Phosphorylation of Gal4p does not affect its DNA binding (14). In the Gal4p/UAS_G crystal structure, the major groove in the central 11 bp of the UAS_G remains quite accessible (10). Thus, another protein could bind to the UAS_G with Gal4p (10), or bind to Gal4p itself, and increase Gal4p/UAS_G affinity. A factor called Egd1p was found to restore the ability of a purified Gal4p-Gal80p complex to bind UAS_G DNA (15). Cells lacking Egd1p showed impaired ability to induce *GAL* expression when pregrown in glucose, where Gal4p is not on the UAS_G, consistent with increased difficulty in Gal4p/UAS_G recognition in the absence of Egd1p.

Transcription activation/Gal80p binding

Transcription activation and Gal80p binding share the same region on Gal4p but are mutationally separable. Gal4p possesses two domains (see Fig. 2) that can activate transcription (1, 2). Both are considered prototypical acidic activation domains. AR2, which is probably the major one (2), also contains the Gal80p interaction region (see Fig. 2), the contacts by which Gal80p inhibits Gal4p function (1, 2). Although sharing the same region on Gal4p, transcription activation and the Gal80p interaction are mutationally separable functions (16). For example, there are constitutive mutants, or 4^C, that contain a Gal4p which is capable of full transcription activation but is insensitive to Gal80p inhibition. In several of these, single amino acid changes were found within the Gal4p carboxy–terminus, residues 859–868 (16).

The transcription activation and Gal80p interaction functions may use opposite faces of a β-sheet region near the carboxy terminus of Gal4p; the acidic residues in this region are dispensible for transcription activation. DNA encoding residues 854–874 in the Gal4p carboxy–terminal region was extensively mutagenized, and this DNA library was used to express mutant Gal4ps from a *GAL4* promoter on a single copy plasmid, to mimic wild–type abundance, in a strain deleted for *gal4* (17). Based on the pattern of mutational effects obtained from a number of these sequenced mutants and on computer structural analysis, it is suggested that amino acids 856–869 form an antiparallel β-sheet structure, with the Gal80p interaction using one face of the sheet and the transcription activation function the other (17). Circular dichroism (CD) confirms the presence of β-sheet in a protein fragment, residues 841–874, containing this region (18). Prior to this work, acidic activation domains like this one have been thought to be amphipathic α-helices or unstructured. In fact, the amphipathic helix

(AH) used extensively in GAL expression studies does not activate transcription when present at the abundance levels of wild–type Gal4p (17) and yields a CD spectrum different from the natural Gal4p fragment (18).

The six acidic residues in the carboxy–terminus have been thought to be crucial for transcription activation. However, mutant Gal4p lacking four, or all six, could still activate transcription at full or significant (~40%) levels (17). Loss of acidic residues 862 and 863 specifically disrupted the Gal80p interaction. Engineered Gal4p with positively charged carboxy–terminal domains were as effective in transcription activation as Gal4p with acidic domains. Acidic residues may simply provide charged determinants to increase accessibility of the hydrophobic residues, the crucial ones for transcription activation (17). Thus, basic residues can work just as well (17).

The Gal4p-Gal80p complex

A Gal4p–Gal80p complex is present in all carbon sources and may be the predominant form of Gal4p in vivo. Gal4p-Gal80p probably interact in all carbon sources; very similar Gal4p-Gal80p complexes can be purified from cells grown in glucose (13) or galactose (14, 19), and in vivo, Gal4p-Gal80p are known to be contact in glycerol and in galactose (1, 2). In the purified complex, Gal4p-Gal80p are bound stoichiometrically with strong affinity, $K_d \sim 5 \times 10^{-9}$ M (13, 19). Gal80p probably is in excess over Gal4p in all carbon sources. It is at least 10-fold more abundant than Gal4p in glucose (9), and *GAL80* mRNA is at least 8-fold more abundant than *GAL4* mRNA in glycerol or galactose (20, 21). This persistent Gal80p excess and the strength of the Gal4p-Gal80p interaction make it likely that most or all of the Gal4p present in vivo will be constitutively bound to Gal80p. In agreement, intact Gal4p has been purified only in a complex with Gal80p, even from strains overexpressing *GAL4*, but Gal80p can readily be purified alone (2).

The apparent scarcity of Gal4p may reflect its presence (with Gal80p) in a large, insoluble complex in vivo. Twenty-two Gal4p molecules will be needed to bind the known UAS_G elements, based on complete occupation of each UAS_G in galactose (1, 5, 6) by two Gal4p (2, 10). However, biochemical approaches estimate one or fewer copies of Gal4p per wild–type cell (1, 13). Gal4p may be part of a large insoluble structure in vivo—perhaps the nuclear matrix and/or a large transcription complex—making it difficult to free/purify and explaining its apparent paucity (13). From the arguments in the previous paragraph, it is likely that Gal4p in this larger structure is present as Gal4p-Gal80p. The inability to isolate Gal4p from cells lacking Gal80p (13) suggests that Gal80p and the insoluble structure might compete for Gal4p and that the Gal4p that can be isolated is "solubilized" by Gal80p.

Gal4p contains a direct glucose response region. Gal4p contains three domains (Fig. 2) that can mediate a direct glucose inhibition of Gal4p (22). An adjacent glucose response domain (GRD) prevents inhibitory domain (ID) function when glucose is absent. Without the GRD, the ID inhibit constitutively.

Gal3p

Gal3p mediates the rapid induction triggered by galactose, an ability it shares with Gal1p, a structural gene product. Gal3p is crucial to the rapid galactose-induction process; induction in wild–type cells occurs within minutes but requires 3 to 5 days in *gal3* mutants (1). Gal3p contains sequence similarities to regions of galactokinase (Gal1p), the only resemblance to known sequences detected in Gal3p (23). Gal3p cannot carry out a galactokinase reaction, but constitutive expression of *GAL1* can restore rapid inducibility in *gal3* mutants, suggesting that Gal1p contains a Gal3p-like activity (2). This activity is distinct from the kinase activity of Gal1p.

STRUCTURAL GENE EXPRESSION/REGULATION

Glucose

Glucose presence triggers a global system that strongly represses many yeast genes (1, 2). On *GAL* genes this repression involves several pathways (2).

a. *Gal4p is absent from the UAS_G in glucose, reflecting a glucose-dependent decrease in Gal4p levels*

The absence of Gal4p from the UAS_G disables the only known transcription activation pathway for *GAL* structural genes. This absence reflects a global change because it is also observed on glucose-insensitive genes like *GAL80*. *GAL4* mRNA levels are lowest in glucose (24); expression of a *GAL4-CAT* hybrid gene integrated into the chromosome at the *GAL4* locus is repressed four- to sevenfold (25). The decreased Gal4p levels resulting from this transcriptional repression are probably sufficient to abolish Gal4p/UAS_G binding (25). Overproduction of Gal4p in glucose restores UAS_G occupation (5) and results in structural gene expression (1).

b. *Gal80-dependent effects are part of repression in glucose*

Galactose is required to activate the pathway that relaxes Gal80p inhibition of Gal4p (1, 2). Thus, in glucose this inhibition remains in place.

c. *URS_G-mediated effects*

A sequence, the URS_G, which can implement glucose repression on a reporter gene, was identified upstream of *GAL1 (2)*, between the UAS_G and the *GAL1* TATA (elements A–C, see Fig. 3*A*). URS_G function depends on the products of several genes associated with general glucose repression (2), including Mig1p, which binds to the URS_G in vitro (2, 26). No URS_G are found upstream of *GAL10* or *GAL7* (27), even though these genes are as strongly glucose–repressed as *GAL1*, suggesting that the URS_G mechanism may be specific to *GAL1*.

Two recent papers have quantified the relative contribution of each of the above–mentioned three mechanisms to repression in glucose (28, 29). Mechanism a alone produces significant (25-fold) repression (**Table 3**), allowing

TABLE 3. *Glucose repression mechanisms*[a]

Wild type	a only (*GAL4*-dep)	b only (*GAL80*-dep)	c only (URS_C-dep)
<0.2%	3%	8%	37%

[a] *GAL1-lacZ* reporter gene expression in glucose as % of wild type expression in galactose (29).

GAL1-lacZ reporter gene expression at only a few percent of full wild-type induced levels. Mechanism b alone produces 13- to 20-fold repression and the URS_C-mediated effect alone produces only a 2- to 3-fold repression. Based on their individual efficiencies, the combination of these three mechanisms can account completely for the 1000-fold repression of *GAL1* expression in glucose, suggesting they are the major, and perhaps only, mechanisms needed to implement full glucose repression (29). Mechanisms a and c are glucose-specific; they depend on factors associated with general glucose repression like Mig1p, which plays a prominent role in both mechanisms. Because the strong mechanisms a and b act concurrently, both must be disrupted, e.g., in a double mutant like *mig1/gal80* (26), to obtain significant structural gene expression in glucose.

Glycerol

In glycerol, the GAL structural genes are inactive but poised for induction, with Gal4p on the UAS_G and Gal3p present to mediate induction if galactose becomes available. In glycerol, only the Gal80p-dependent inhibition of Gal4p prevents *GAL1*, *-2*, *-7*, and *-10* transcription. Thus, disruption of *GAL80* results in extremely high–level structural gene expression, even higher than wild–type induced levels (**Table 4**; 30). The basic mechanism (or mechanisms) of Gal80p inhibition of Gal4p may be similar in glycerol or glucose. The inhibition is certainly not glucose-dependent because Gal80p alone inhibits *GAL* expression more effectively in glycerol (1000-fold) than in glucose (13– to 20-fold; see Structural Gene Expression/Regulation). The level of *GAL80* expression is the same in glucose or glycerol, consistent with Gal80p carrying out the same function in both carbon sources. *MEL1* is expressed in glycerol, in contrast to *GAL1*, *-2*, *-7*, and *-10*. This leaky regulation may reflect the origin of *MEL1*, from related yeasts (31), or its unique role in galactose utilization, as a secreted galactosidase.

GAL structural genes are poised for rapid activation in glycerol. Induction can occur within minutes in glycerol-grown cells, but requires hours in glucose-grown cells (1). This rapid inducibility reflects the presence of Gal4p on the structural gene UAS_G elements, which allows expression to be triggered by a conformational change in a promoter-bound complex, and depends on the presence of the induction mediator Gal3p. This poised state might have evolved to allow cells a quick response to galactose availability when growing in poorer carbon sources like glycerol.

Galactose

We will discuss the fully induced state, reflecting long-term growth in galactose, and the induction process triggered by galactose addition to uninduced (glycerol-grown) cells.

Galactose entry

In galactose-grown cells, galactose transport occurs by a low-affinity and a high-affinity process (32), both of which depend on the *GAL2* gene product, a facilitated-diffusion galactose transporter (32) associated with the cell membrane (33). The high-affinity process also depends on Gal1p (32). The initial entry of galactose into cells growing in glycerol may occur by a constitutive, *GAL2*-independent process (34) or by Gal2p-mediated entry, enabled by low-level *GAL2* expression in glycerol (5).

The galactose signal

Gal3p appears to act like a direct transducer in the induction pathway. Gal3p was thought to mediate the galactose-dependent process that releases Gal80p inhibition of Gal4p by making a small molecule inducer from galactose (1). However, in vitro studies could not find such a molecule (2) and it has recently been suggested that Gal3p itself may trigger the change that releases Gal80p inhibition (2, 35), perhaps by a direct interaction with the Gal4p-Gal80p complex. This is proposed to involve a galactose-activated form of Gal3p (35). Activation seems necessary because Gal3p is present in glycerol-grown cells, but does not trigger induction. Galactose also induces expression of *GAL3* (23),

TABLE 4. *Structural and regulatory gene expression*[a]

Gene	Glucose		Glycerol		Galactose	
	WT	*gal80*[b]	WT	*gal80*[b]	WT	*gal80*[b]
GAL1	<0.3%	3%	<0.3%	300%	100%	150%
GAL80	10-20% (*GAL4*-indep.)		10-20% (*GAL4*-indep.)	100%[c]	100% (*GAL4*-dep.)	
GAL4	30-50%		200%		100%	

[a] As % of wild type (WT) expression in galactose for that gene (*GAL1* (30); *GAL80* (21); *GAL4*, glucose versus glycerol (25), glycerol versus galactose (24))
[b] gal80-disrupted strain, isogenic with wild type (30) [c] 4^C constitutive mutant (21).

but induction of the structural genes does not require protein synthesis (1) and so cannot depend solely on *GAL3* transcriptional activation. Because the Gal4p-Gal80p complex is already present on the UAS_G elements of the genes that will be activated, the change in the complex triggered by Gal3p (activity) must occur in the nucleus. Thus, a signal of galactose presence must be conveyed there from the cytoplasm. Galactose, Gal3p, and/or a specific transduction pathway may perform this function.

Gal1p can also mediate induction via its Gal3p-like activity (2, 35). However, *GAL1* is not expressed in glycerol, so the initial induction triggered by galactose must be Gal3p-mediated. As induction proceeds, Gal1p becomes increasingly available and can be the major factor in maintaining the induced state (36). Because *GAL1* is one of the genes induced, Gal1p induction ability could result in autoinduction, driving its own expression to ever higher levels. *GAL80* disruption allows increased *GAL1* expression in galactose (Table 4; 30), suggesting that Gal80p normally regulates the induced level of *GAL1* expression, perhaps to temper *GAL1* autoinduction.

The yeast *Kl. lactis* also has a Gal1p with induction plus galactokinase activities (36), but no *GAL3*. Thus, *Kl. lactis* Gal1p must mediate induction alone, and like *S. cerevisiae* Gal3p, *Kl. lactis* Gal1p is present in glycerol-grown cells to trigger induction. The ability of *Kl. lactis* to dispense with Gal3p may reflect an adaptation to its normal carbon source (36).

The target of Gal4p activation is probably a protein (or proteins) in the basal initiation complex

The ability of Gal4p to activate transcription throughout eukaryotes suggests that it contacts a universal target (or targets) such as a general transcription factor (2). Effects on TFIID/DNA interactions (2), on TFIIB assembly into a complex (2), and on TFIIA-TFIID complex assembly (37) have been observed in vitro using heterologous, UAS_G-containing promoters and artificial Gal4p derivatives.

The novel structure of the Gal4p carboxy terminus, with transcription activation and Gal80p interaction on opposing faces of a β-sheet (17), may indicate that Gal80p modulates the interaction between the activation face of Gal4p and its target. For example, in 4^c mutants, which lack the Gal80p interaction with Gal4p, galactose-induced expression can occur at even higher levels (two– to fourfold) than in fully induced wild type (17), suggesting that the Gal4p activation face can interact more strongly or more efficiently with its target when the Gal80p interaction is absent. In 4^e mutants, expression in glycerol can reach the same levels of hyperactivation as in galactose. This suggests that Gal4p-Gal80p location/disposition with respect to the activation target may not be grossly different in the two carbon sources, raising the possibility that Gal80p modulation of the activation face of Gal4p might differ in degree between glycerol and galactose, but not in its basic nature. Gal4p-Gal80p contact probably exists within a larger insoluble structure (see Background); the activation target of Gal4p may be part of this larger structure. It is striking that this Gal80p-mediated control can be completely destroyed by single amino acid changes (16, 17).

Gal11p and Swi/Snf proteins are subsidiary activating factors for GAL structural gene expression

Induced levels of *GAL1* expression are reduced 3- to 10-fold in *gal11* mutants (2). Gal11p may help Gal4p interact with the general transcription machinery (38); Gal11p increases basal transcription in vitro, by enhancing preinitiation complex formation (39), and is part of a complex that can mediate activator-driven transcription in a defined in vitro system (40). Gal11p is suggested to be a special kind of basal transcription factor (39), and consistent with this suggestion, it is not *GAL*-specific (2, 38). However, Gal11p acts selectively on *GAL* genes; its loss affects *GAL1* or *GAL2* expression but not *GAL80* or *MEL1* expression (2).

The global activators Swi1, Swi2, and Swi3, and Snf2 (Swi2), Snf5, and Snf6 are involved in *GAL* expression (41, 42). Swi-Snf proteins may assist directly in transcription activation and/or alter chromatin structure on gene promoters (42). Gal4p might be a good activator because it effectively recruits or interacts with auxiliary factors like Gal11p/Swip/Snfp.

Gal4p phosphorylation

Gal4p shows two phosphorylated forms, $Gal4_{II}$ and $Gal4_{III}$ (43). $Gal4_{III}$, the most highly phosphorylated form, is associated with the most active *GAL* expression (43), $Gal4_{II}$ reflects transcriptional competence and unphosphorylated Gal4p is transcriptionally inert (14, 43). $Gal4_{II}$ and unphosphorylated Gal4p bind equally well to DNA and to Gal80p in vitro (14). The precise role of Gal4p phosphorylation is unclear.

REGULATION OF THE *GAL*-SPECIFIC REGULATORY GENES

The *GAL*-specific regulatory genes are expressed in carbon sources besides galactose (Table 1, Table 4). These expression patterns plus promoter analyses can provide insights on the function and control of these regulators.

Glucose

GAL4 is repressed in glucose by global, not GAL-specific, mechanisms; GAL80 is not repressed at all. GAL4 has no UAS_G (Table 1) and is not regulated by itself or by Gal80p (1). Glucose repression of *GAL4* involves primarily global control; it is mediated by DNA sequence elements that resemble a glucose repression element found on non–*GAL* genes, and most of the gene products that implement *GAL4* repression are general factors that help repress many gene sets (25). The repression elements on *GAL4* lie between the transcription start site and upstream activating sequences. Mig1p has been shown to bind in vitro to the most upstream repression element (26) and thereby implement *GAL4* repression (29), either by interfering with assembly of tran-

scription machinery on the activating elements (25) or blocking its access/movement to the transcription start site.

GAL80 expression is not reduced at all in glucose, so neither global nor *GAL*-specific glucose repression mechanisms affect *GAL80*. This expression is *GAL4*-independent (21). *GAL3* is completely glucose-repressed, by unknown mechanisms.

Glycerol

All three regulatory genes are expressed in glycerol. GAL4 mRNA levels are highest in glycerol (24; Table 4). However, even these maximal levels are quite modest, estimated at <1 transcript per cell (1, 24). The scarcity of *GAL4* mRNA is due to the inherent weakness of the *GAL4* promoter in activating transcription, not to posttranscriptional mRNA instability (20). The weak *GAL4* promoter is unique (20). Just upstream of the glucose repression elements lie upstream essential sequences (UES) which are required for any *GAL4* expression. TATA elements cannot substitute for the UES nor can it functionally replace a TATA. Upstream from the UES are activating sequences that boost *GAL4* expression above the basal level. These sequences are not UAS_G elements; their function on *GAL4* cannot be carried out by a structural gene UAS_G nor can they substitute for a UAS_G to drive heterologous expression.

GAL80 expression in glycerol is *GAL4*-independent and occurs at the same basal level as in glucose (21). *GAL80* expression thus might be driven by the same Gal4p-independent promoter in both carbon sources. In mutants lacking Gal4p function, *GAL80* expression in galactose drops to this same basal level (21), suggesting that this basal promoter acts constitutively. It has not been identified. In 4^C mutants, where Gal80p inhibition of Gal4p is disabled (see Specific Regulatory Factors), *GAL80* expression in glycerol rises to the Gal4p-dependent levels seen in galactose (21; Table 4). Thus, Gal80p must inhibit UAS_G/Gal4p-mediated activation on its own gene in glycerol. This probably involves the same mechanism (or mechanisms) used by Gal80p to inhibit structural gene expression in glycerol.

Galactose

The expression of GAL3 and GAL80 is modestly induced in galactose, via a UAS_G and Gal4p; GAL4 expression is not induced. Galactose induces *GAL3* expression 3- to 5-fold (23) and *GAL80* expression 5- to 10-fold (21) above their expression levels in glycerol. Induced expression is *GAL4*-dependent and glucose-repressible and Gal4p is bound strongly to the *GAL80* UAS_G in galactose (6). Induced *GAL80* and *GAL3* expression must be promoted from the UAS_G by Gal4p, like induced structural gene expression.

It is noteworthy that the same promoter and activator, the UAS_G and Gal4p, can promote both modest (*GAL3, -80*) and strong (structural genes) induction. The modest induction of *GAL80* is probably not due to weakness of its UAS_G, because when removed from *GAL80* this element can promote expression as well as either of the *GAL7* UAS_G elements, each of which promote expression at nearly full structural gene induction levels (44). The weaker induction of *GAL80* may reflect negative control or a change in the role of auxiliary factors like Gal11/Swi/Snf. For example, *gal11* loss has little or no effect on *GAL80* expression (2).

How GAL regulatory gene control might implement structural gene regulation

The activator (*GAL4*) and the negative regulator (*GAL80*) are constitutively expressed at low levels. These vary a fewfold with carbon source (Table 4). Small variations in *GAL4* expression can bring about very large changes in structural gene expression via the concentration-sensitivity of Gal4p/UAS_G binding and by utilizing protein-protein (conformational) changes (45). For example, low Gal4p levels in glucose, reflecting ~5-fold reduced *GAL4* expression (Table 4), produce the major facet of repression, nonoccupation of the UAS_G by Gal4p. In glycerol, *GAL4* mRNA levels are highest (Table 4), perhaps to produce enough Gal4p to ensure occupation of the structural gene UAS_G elements, a major feature of this poised state, despite a lower Gal4p/UAS_G affinity. For proper regulation in glycerol and glucose, there must be enough Gal80p to bind to and inhibit the Gal4p activation domain. The *GAL80* basal promoter simply provides Gal80p in excess of the levels needed for this function. The lower Gal4p/UAS_G affinity in glycerol results in a weaker Gal4p/UAS_G interaction on *GAL80*; this may minimize interference with the activity of the basal *GAL80* promoter in this carbon source. In galactose, Gal4p can activate structural gene expression because the inhibitory function of Gal80p is relaxed. This involves a conformational change in a promoter-bound, constitutive Gal4p-Gal80p complex. Thus, structural gene activation in galactose does not depend on increased expression of Gal4p activator. The increased expression of the negative regulator in galactose may reflect a new role for Gal80p, tempering structural gene expression by controlling structural gene upstream region chromatin structure (see Chromatin Structure and *GAL* Gene Expression). This increased *GAL80* expression in galactose is mediated by the same elements that mediate induction of *GAL1* and the other structural genes, the UAS_G and Gal4p, thus obligatorily linking the two processes (6).

Gal3p differs from the other two *GAL*-specific regulators: it is not constitutively expressed and its function is not unique (shared with Gal1p). The properties of Gal3p are more like those of a signal transducer than a regulator. Gal3p allows the most efficient galactose-induction process in *S. cerevisiae*, perhaps reflecting conditions in which this yeast evolved.

CHROMATIN STRUCTURE AND *GAL* GENE EXPRESSION

Chromosomal UAS_G are located within nonnucleosomal hypersensitive regions flanked by positioned nucleosomes. The *GAL1-10* UAS_G lie within an ~170 bp region (**Fig. 3**), which is nonnucleosomal in all carbon sources (1); the

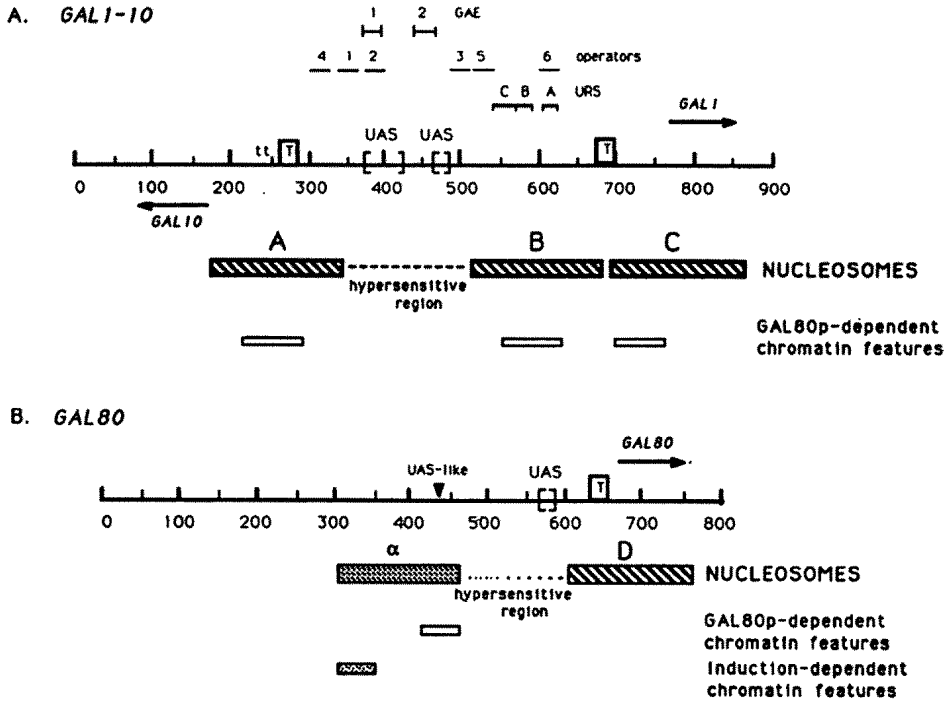

Figure 3. Sequence organization and chromatin structure of *GAL1-10* and *GAL80*. The *GAL1-10* and *GAL80* DNA sequence features discussed in the text are located to scale on and above the thick solid line (UAS = UAS_G, T = TATA). The numbers are base pairs (bp) measured from the EcoRI site in *GAL10* or from an MboII site upstream of *GAL80*. Directions of *GAL10*, *GAL1*, and *GAL80* transcription are shown by arrows. Below the line the chromatin features are located to scale for comparison to the sequence features. UAS-like refers to a sequence with partial but significant similarity to a typical UAS_G element.

entire region is always uniformly and highly hypersensitive and it never produces the characteristic 10 bp nucleosomal ladder from DNase I digestion (46), as do the regions around it. Thus, Gal4p does not have to compete with nucleosomes to bind to the *GAL1-10* UAS_G in vivo. Nucleosomes must be kept off the hypersensitive region by specific mechanisms, such as a factor that binds within the region (2, 47), because in vitro this DNA associates avidly with histones (48). The single *GAL80* UAS_G also lies within a constitutive, ~150 bp, nonnucleosomal hypersensitive region (6; Fig. 3), suggesting that this type of organization may be a general *GAL* gene feature.

In the noninduced state, positioned nucleosomes (A–D in Fig. 3) lie between the hypersensitive regions and the genes, in the single chromosomal copy (6, 46, 49) or in multicopy *GAL1-10*-containing plasmids (47). The *GAL10* TATA, the *GAL1* TATA/transcription start site, and the *GAL80* TATA/transcription start site lie within these positioned nucleosomes. Nucleosome B contains the URS_G/Mig1p binding site. Positioning of nucleosomes A–C is thought to be imposed by the factor that binds within the hypersensitive region (47), but *GAL80* lacks the factor binding sequence. Thus, other mechanisms must determine the positioning of nucleosome D. The distal *GAL80* nucleosome α (Fig. 3) is nonpositioned (6).

Galactose induction causes disruption of the upstream positioned nucleosomes by a GAL4-dependent process; gene inactivity causes their replacement by a process dependent on GAL80. Galactose-induction produces in vivo structural changes on the *GAL1* TATA/nucleosome B region and on the *GAL10* TATA (50). The *GAL1* changes are *GAL4*-dependent and involve the disruption of nucleosome B (51). In the multicopy *GAL1-10* templates, DNA became more accessible to cleavage under induction conditions but retained some nucleosomal character (52), perhaps reflecting structural heterogeneity among the multiple templates. Recent analysis of the single chromosomal copy (D. Lohr and J. Lopez, unpublished results) has shown that gene activation causes *GAL4*-dependent disruption of nucleosomes A–C on *GAL1-10* and nucleosome D on *GAL80*. Transcription-associated DNA melting on *GAL1-10* begins ~20 bp downstream of the *GAL1* and *GAL10* TATA elements (53), in regions uncovered by the nucleosome disruption. In addition to freeing this DNA from histone constraints, nucleosome disruption might facilitate the DNA melting in this region by liberating the negative supercoiling constrained in the nucleosomes. It is unclear whether the *GAL4*-dependence of nucleosome disruption reflects the direct action of Gal4p or indirect action through factors like SWI/SNF (54). In artificial constructs, Gal4p can disrupt or displace a UAS_G- bound nucleosome (see ref 55), but the relevance of this activity to events on the *GAL* genes, where Gal4p carries out nucleosome disruption at distant sites while bound to the UAS_G, is unclear. Induction does not cause the disruption of α, the distal *GAL80* nucleosome (Fig. 3).

Under inactivating conditions, nucleosomes A–D quickly reappear on the same *GAL1-10* and *GAL80* regions from

which they were displaced during activation (D. Lohr and J. Lopez, unpublished results). This replacement is *GAL80*-dependent. Thus, the activator promotes expression-associated nucleosome removal, whereas the negative regulator functions in the reverse process.

Upstream nucleosomes may play a role in GAL regulation. Nucleosome presence on the TATA/start site regions denotes the gene inactive state and nucleosome loss, produced by histone depletion, allows *GAL1* expression under normally nonactivated conditions (56). Thus, the presence of these nucleosomes might in itself have a regulatory role (57). However, removal of only the amino–terminal tails from histone H4 actually reduces the level of induced *GAL1* expression ~20-fold (58). These H4 tails may be the site through which Gal4p implements nucleosome disruption during induction; in their absence, nucleosome disruptability and thus induction are affected. Gal80p affects the structure (49) and mediates the occupancy (see above) of *GAL1-10* upstream nucleosomes A–C. Thus, Gal80p might interact with them, directly or via an adapter. The loss of Gal80p function (30) or removal of the amino–terminal tails of histone H3 (59) each results in roughly similar levels of *GAL1* hyperexpression in galactose. Thus, the Gal80p contact site on these nucleosomes might be their H3 tails. The loss of this interaction site could restrict the ability of Gal80p to place or keep nucleosomes on these regions, explaining the hyperexpression observed. Based on these ideas, we suggest that nucleosome occupation of these TATA/start site regions might reflect the continuous competition of Gal4p-dependent nucleosome disruption and Gal80p-dependent nucleosome replacement. Activation conditions (signals) favor (and foster) disruption; inactivity, or simply a lack of activation signals, favors replacement. Such a competition fits the observations that the degree of nucleosome B disruption is proportional to the strength of the Gal4p activator (51) and that nucleosome disruption/replacement processes are quite rapid (D. Lohr and J. Lopez, unpublished results). The Gal80p modulation of induced *GAL1* expression levels (see Structural Gene Expression/Regulation) might be implemented by the Gal80p-dependent maintenance of at least partial nucleosome presence on these upstream regions in galactose, and thus also reflects this competition. At the 5′ end of the *GAL* genes there must be a large regulatory nucleoprotein complex containing *GAL*-specific and global factors (GAL11p, Swip, Snfp) and the basal initiation factors. A future task is to integrate the location, structure, and activity of this complex with the nucleosome structure within the chromosomal context. FJ

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